Controlling Prostate Cancer Through Durable Inhibition of Androgen Receptor Activity

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Abstract

The large majority of clinical prostate cancers remain dependent on androgen receptor (AR) activity for proliferation even as they lose their responsiveness to androgen deprivation or antagonism. AR activity can be maintained in these circumstances by: increased AR synthesis – often reflecting increased NF-kappaB activation; up-regulation of signaling pathways which promote AR activity in the absence of androgens; and by emergence of AR mutations or splice variants which render the AR constitutively active. Drugs targeting the N-terminal transactivating domain of the AR, some of which are now in pre-clinical development, may have greater potential for achieving durable inhibition of AR activity than do currently available anti-androgen agents. Concurrent measures which suppress AR synthesis or boost AR turnover could be expected to complement the efficacy of such drugs. A number of nutraceuticals which show efficacy in prostate cancer xenograft models – including polyphenols from pomegranate, grape seed, and green tea, the crucifera metabolite diindolylmethane, and the hormone melatonin –have the potential to suppress AR synthesis via down-regulation of NF-kappaB activity; clinical doses of salicylate may have analogous efficacy. The proteasomal turnover of the AR is abetted by diets with a high ratio of long-chain omega-3 to omega-6 fatty acids – which likewise are beneficial in prostate cancer xenograft models. Hence, it is proposed that a regimen combining an N-terminal domain-targeting drug, various nutraceuticals/drugs which down-regulate NF-kappaB activity, and supplemental fish oil, has great potential for blocking proliferation of prostate cancer by targeting its characteristic addiction to androgen receptor activity.

How Prostate Cancer Becomes Androgen Independent

The large majority of prostate cancers – including those that are no longer responsive to androgen deprivation measures, and thus are characterized as androgen-independent or androgen-refractory – are dependent on the transactivating activity of the androgen receptor (AR).[1-3] In prostate epithelium and most prostate cancers, AR-mediated transcription is required for G1-S cell cycle transition; absence of AR activity is associated with suppression of G1 cyclin expression and increased levels of cyclin-dependent kinase inhibitors.[4, 5] A small minority of prostate cancers – which fail to express PSA - somehow manage to maintain proliferation despite methylation-mediated silencing of the AR gene.[6] In prostate cancers that relapse during androgen deprivation, a marked up-regulation of AR mRNA is usually observed; amplification of the AR gene occasionally accounts for this, but more commonly this stems from accelerated transcription of the AR gene.[2, 7-10] It is presumed that a sufficient increase in the number of androgen receptors can compensate for a reduction in androgen level. In part, this reflects the fact that it is not feasible to reduce the level of androgens to zero in the prostate, as weak androgens of adrenal origin such as DHEA may interact with the AR.

Moreover, various tyrosine and serine/threonine kinases, via phosphorylations of the N-terminal domain of the AR or of its coactivators, can promote the intranuclear uptake and transactivational activity of the

AR, independent of androgen binding; these include Src (activated via EGFR), Ack1 (activated via HER2/HER3 receptors), and MAP kinase.[11-17] Intranuclear Stat3 can boost AR transactivational activity via direct binding to the N-terminal domain.[17] Akt can increase AR activity by recruiting beta-catenin to the nucleus as a coactivator, and has the potential to disinhibit AR activity by excluding FOXO1 from the nucleus.[18-24] Increased expression of the coactivators required for AR activity is also commonly encountered in castration-resistant cancers.[25, 26] It seems likely that achievement of "androgen independence" often reflects both an increase in expression of AR – and possibly of its coactivators – as well as an up-regulation of kinase activities that can directly activate or potentiate AR activity.

Alterations of AR structure can also contribute to androgen independence. Mutations of the ligand-binding region of the AR may arise which make it more responsive to weak androgens or other steroid hormones, turn androgen antagonists such as bicalutamide into agonists, or which render the AR constitutively active.[26-29] Moreover, recent research has established that advanced androgen-insensitive prostate cancers commonly express splice variants of the AR which lack the ligand-binding domain, but nevertheless possess constitutive transactivating activity.[30-33] Evidently, no degree of androgen deprivation will abolish AR activity in prostate cancers expressing AR variants that are constitutively active in the total absence of androgens.

Drug Targeting of the Androgen Receptor N-Terminal Domain

For that reason, drugs which target the N-terminal domain of the AR receptor, responsible for its transactivating activity, would appear to have greater potential for durable inhibition of AR activity than measures which target the C-terminal ligand-binding region, which demonstrably is not essential for AR activity.[34, 35] Such agents also have the potential to block the interaction of the AR with activating kinases targeting the N-terminal domain. (Theoretically, drugs targeting the DNA-binding domain could also be durably effective, but this domain is so homologous to that of various other steroid receptors that it would be difficult to find such a drug that inhibited only the AR.[36]) Sadar and colleagues, following this line of reasoning, have characterized several agents with high affinity for the N-terminal domain of the AR that impair its interaction with essential coactivators and hence block its transactivational activity. EPI-001, a naturally occurring chlorinated derivative of the industrial chemical bisphenol A diglycidyl ether (BADGE), is one of these agents; this agent inhibits AR activity in all prostate cancer cell lines tested, and moreover induces shrinkage of human androgen-independent prostate cancers implanted in nude mice, in doses that are not overtly toxic.[36] Chlorinated peptides, known as sinkotamides, derived from a marine sea sponge, likewise target the N-terminal domain of the AR and inhibit its activity.[37] It remains to be seen whether the AR N-terminal domain can mutate in ways that markedly lessen the binding affinity of these agents while preserving its ability to interact appropriately with key coactivators; if such mutations are rare or impossible, these agents may possess durable anti-androgenic activity.

Nonetheless, it is quite conceivable that feasible and tolerable concentrations of drugs targeting the N-terminal domain of the AR may fail to achieve a total elimination of AR activity. And, not unlikely, some prostate cancers may increase their expression of membrane transport proteins which can evict these drugs from the cell. In these cases, increased AR expression would represent an escape mechanism for the cancer, as it does when prostate cancers are treated with anti-androgen drugs. Therefore, the concurrent use of adjuvant measures which suppress the accelerated transcription of the AR gene would seem likely to aid therapeutic outcomes.

Down-Regulating NF-KappaB Activity to Suppress Androgen Receptor Synthesis

Undoubtedly, there are a variety of mechanisms are responsible for the increase in AR expression observed in androgen-insensitive prostate cancers. However, there is good reason to suspect that a constitutive increase in NF-kappaB activity, also commonly observed in androgen-insensitive prostate cancers, and a negative prognostic sign, is the chief reason for the increase in AR expression.[38-44] The promoter region of the AR gene possesses NF-kappaB response elements, and several studies show that stimulation of NF-kappaB activity increases AR levels in prostate cancer cells, and stimulates reporter genes containing the AR gene promoter.[43, 45-48] This phenomenon appears to reflect direct binding of NF-kappaB to the AR promoter. Hence feasible measures which suppress NF-kappaB activation in androgen-insensitive prostate cancer could be expected to decrease AR expression.

Further studies are required to define why NF-kappaB is typically constitutively active in advanced prostate cancer. Several recent investigations have shown that "atypical" forms of protein kinase C (e.g. PKC-zeta, PKC-iota/kappa) are activated in various prostate cancer cell lines, and stimulate NF-kappaB activation, presumably via phosphorylation of IKK-beta.[49-51] These atypical PKCs appear to mediate the activation of NF-kappaB by TNF-alpha in prostate cancer. Increased production of oxidative stress via NADPH oxidase activity would also seem likely to up-regulate NF-kappaB activation in many prostate cancers.[47, 48, 52-59] Akt activation – typical of the many prostate cancers that are PTEN deficient – might also participate in NF-kappaB activation.[60-63]

Intriguingly, certain nutraceuticals which have shown potential for prostate cancer control in cell cultures or xenograft models – such as polyphenols derived from pomegranate, grape seed, or green tea, the crucifera-derived compound diindolylmethane, and the hormone melatonin – have been found to blunt NF-kappaB activation in prostate cancer cell lines, either in vitro or in vivo.[64-76] It would be of interest to define the mechanisms responsible for such inhibition. In addition, feasible clinical concentrations of the drug salicylate are known to suppress NF-kappaB activation, reflecting a direct inhibitory interaction with IKKbeta.[76-80] The phycocyanobilin in spirulina has the potential to blunt oxidative stress-mediated up-regulation of NF-kappaB activity via inhibition of NADPH oxidase complexes.[81, 82] Hence, a regimen compromised of appropriate polyphenols, melatonin, spirulina, and salicylate (best administered as well-tolerated salsalate[83]) may have potential for lessening the NF-kappaB activity of prostate cancers and thereby diminishing their AR expression.

Boosting NF-kappaB Turnover with Omega-3

Theoretically, measures which boost the proteasomal degradation of the AR would likewise have utility in management of prostate cancer. In that regard, there is a recent report that, when mice bearing PTEN-null castrate-resistant prostate cancer were fed a diet with a 1:1 ratio of omega-6 to omega-3 (the omega-3 provided by equal amounts of EPA and DHA), as contrasted with a diet with an omega-6/omega-3 ratio of 40, the expression of AR protein in the cancers was notably diminished, despite the fact that AR mRNA was not decreased.[84] Cell culture studies revealed that long-chain omega-3 fats promoted proteasomal degradation of the AR in these cancer cells. Likewise, a previous study had reported lower levels of AR in prostate cancer cells exposed to EPA and DHA.[85] These findings may help to rationalize other studies in which diets with high omega-3/omega-6 ratio have retarded growth of prostate cancers in mice, as well as epidemiology correlating increased oily fish intake with decreased risk for prostate cancer mortality.[86-95]

Overview

In overview, it is proposed that a regimen consisting of a drug that blocks the transactivational activity of AR by targeting its N-terminal domain, coupled with a range of feasible measures that suppress synthesis of AR by down-regulating NF-kappaB activity (e.g. polyphenols, melatonin, spirulina, salsalate), or that promote proteasomal degradation of AR (e.g. omega-3), may have considerable potential for durable control of prostate cancer. Such a strategy targets prostate cancer's idiosyncratic "Achilles heel" – its addiction to androgen receptor activity. Until such time as N-terminal domain-targeting drugs are clinically available, the ancillary agents cited here, if administered in sufficient doses, should have potential for slowing the onset of androgen independence and prolonging survival.

It should of course be noted that AR activity is a necessary *but not sufficient* condition for the emergence and survival of prostate cancer; therapeutic strategies that down-regulate AR activity can be complemented by measures that target other pathways promoting cancer spread. Quite aside from its impact on AR expression, NF-kappaB activity can work in a number of ways to promote survival and aggressive behavior in prostate cancer; hence, inhibitors of NF-kappaB may not only slow proliferation but also up-regulate apoptosis and lessen invasiveness. Moreover, some of the agents mentioned above have potential for intervening in other signaling pathways – downstream from cytokine or growth factor receptors – that promote survival and aggressiveness in many prostate cancers. (And certain of these signaling pathways increase androgen-independent AR activity via interaction with the N-terminal domain, or via other mechanisms, as discussed above.) Additional agents may have some utility in this regard; of particular interest is the HIV protease inhibitor nelfinavir, which, in a concentration (10 μM) approximately twice the standard clinical plasma level, has been found to decrease activation of both Akt and Stat3 and suppress AR transcription activity in LNCaP human prostate cancer cells.[96] Other feasible adjuvant measures with potential for aiding control of prostate cancer include lycopene, vitamin D, soy isoflavones, metformin, low-dose aspirin, and a low-fat plant-based diet.[97-116]

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